



# Interaction of Chromium with Nickel in the Induction of Sister Chromatid exchanges in Chinese Hamster Ovary cells.

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## Abstract

Epidemiological studies report that populations exposed to complex mixtures show increased incidence rates of cancer. Therefore, chemical interaction is of major concern in the assessment of risk by regulatory agencies. In this study, treatment of CHO (Chinese Hamster Ovary) cells with nickel chloride (in 1.0 and 5.0  $\mu\text{M}$ ), or sodium chromate (0.5, 1.0, and 2.5  $\mu\text{M}$ ) induce sister chromatid exchanges (SCE) in a dose dependent fashion. Statistical analysis of the interaction factor, show that the combined treatments of nickel (1.0 and 0.5  $\mu\text{M}$ ) with chromium (0.5, 1.0 and 2.5  $\mu\text{M}$ ) interacted antagonistically for the induction of SCE. Previously, we reported that nickel with chromium, with UV light and with X-rays interacted antagonistically for the induction of SCE and MN (micronuclei) in human peripheral lymphocytes. These observations indicate that heavy metals, such as nickel and chromium, present in complex mixtures, may reduce the response, even in the presence of strong SCE or MN inducers, and may lead, therefore, to an underestimate of chemical exposure as assessed by these assays. Therefore, further studies are a necessity.

## Introduction

Epidemiological studies and in vivo and in vitro tests indicate that both Ni(II) and Cr(VI) are mutagenic and carcinogenic agents in humans and experimental animals. Ni(II) interferes with normal DNA replication or repair, and its compounds are known to be weak inducers of DNA single strand breaks and DNA-protein cross-links in cultured mammalian cells. Hexavalent chromium is a strong mutagen causing base modification, single and double-strand breaks, and is a carcinogen known to cause lung cancer in humans at a long term exposures.

Both Ni(II) and Cr(VI) are commonly used industrial metals, to which workers are frequently exposed. Ignorance about the decreased potency of the mixture between Ni(II) and Cr(VI) can lead to an underestimate of risk assessment – cytogenetic studies in the lymphocytes of electric welders exposed simultaneously to chromium and nickel indicated that there was little difference in DNA damage between the welders and the non-exposed control.

In this present study, the antagonistic behavior of the two mutagens will be demonstrated through the Sister Chromatid Exchange (SCE) essay, which is a suitable test for monitoring exposures to low levels of chemicals.

## Methods

### Cell Culture and Seeding

CHO (Chinese Hamster Ovary Cells) AA8 cells were cultured in T-25 flasks with Eagle's Minimal Essential Medium (DMEM), and stored in the incubator with 5%  $\text{CO}_2$  at 37°C. 400,000 cells were transferred and seeded in 50mm dishes and incubated for 24hrs.

### Treatment

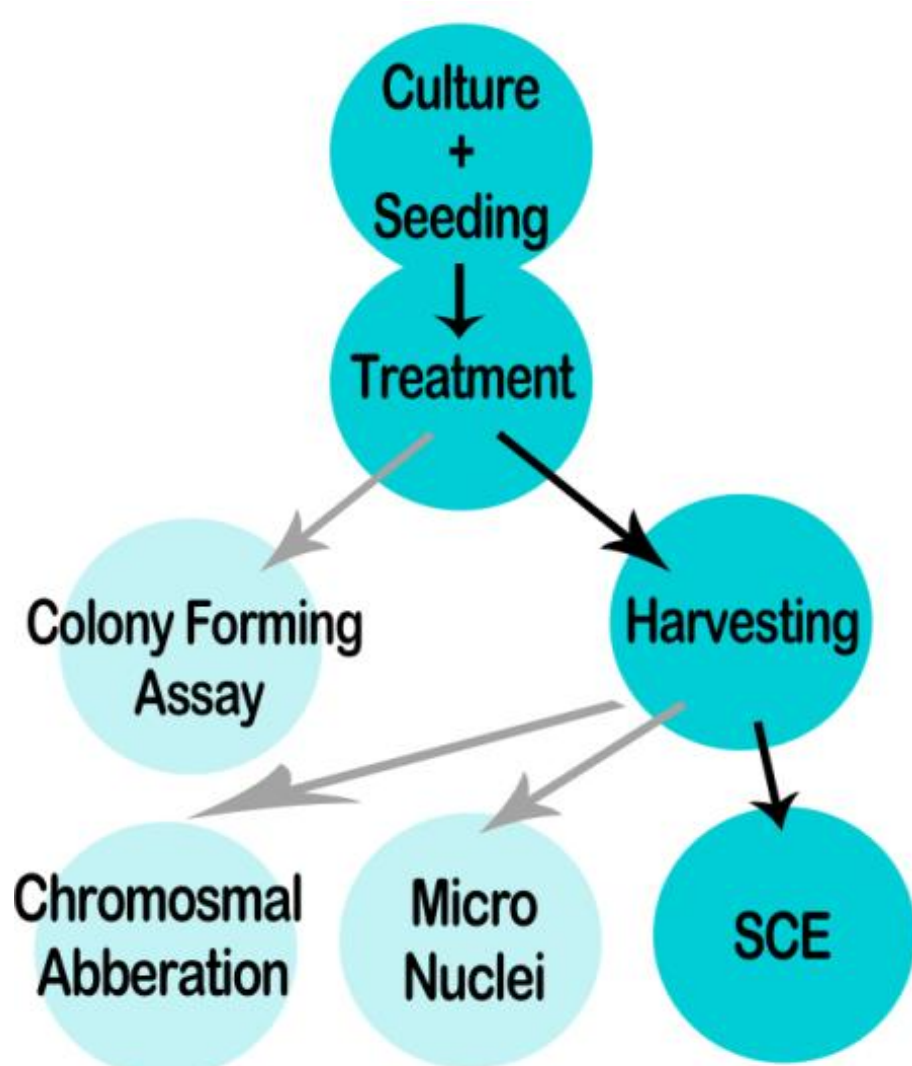
Different concentrations of  $\text{NiCl}_2$  (1.0 and 5.0  $\mu\text{M}$ ) and  $\text{Na}_2\text{CrO}_4$  (0.5, 1.0, 2.5, and 5.0  $\mu\text{M}$ ) were prepared on the day of the treatment, and were applied to the cells seeded 24hrs prior to the treatment. Cultures were treated alone and in combination with the metals along with bromodeoxy uridine.

### Harvesting

After 24hrs of incubation, 0.08  $\mu\text{g/mL}$  of Colcemid was added to each dish and returned to the incubator for another 2 hrs. After the 26hrs of incubation, the cells were trypsinized, centrifuged, and the supernatant was discarded. The cells were treated with 75mM KCl hypotonic solution and centrifuged. The remaining cells were fixed with methanol/acetic acid (3:1), and the slides were prepared.

### Sister Chromatid Differential Staining

Slides were treated with 25  $\mu\text{g/mL}$  of Hoechst solution under long wave UV light for 2-3hours. The slides were then treated with 2X SSC (sodium chloride sodium citrate) buffer and stained with giemsa. Stained slides were coded and randomly scored by a team of two scorers. 30 spread second division metaphases cells with differential staining were scored for each treatment. Each point of breakage and rejoining was counted as one sister chromatid exchange.

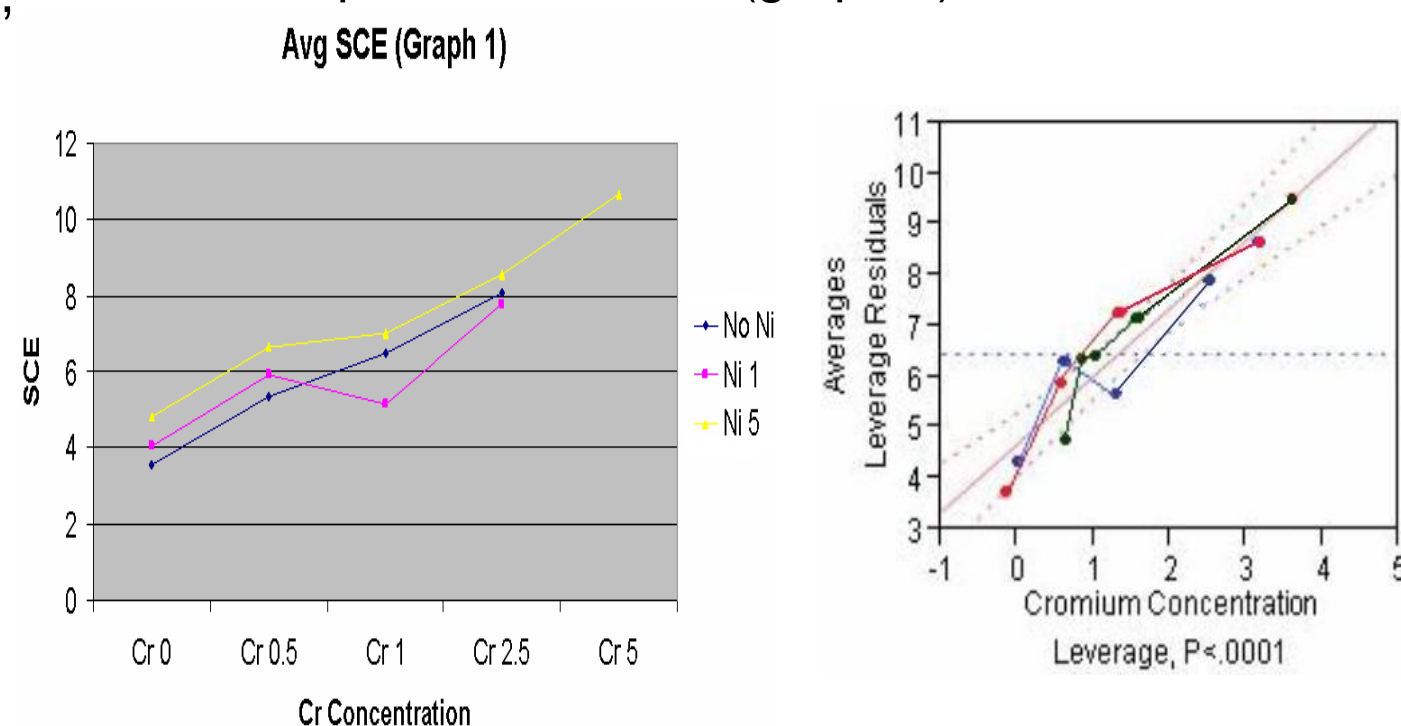


The CHO AA8 cells( donation by Dr. John Wise, Main Center of Toxicology) were treated with various concentrations of  $\text{NiCl}_2$  and  $\text{Na}_2\text{CrO}_4$  solutions. The first set of treatments was implemented with the metals alone, and the induction of SCEs was determined. Treatments with Cr(VI) alone or Ni(II) alone have shown elevated SCE frequencies compared to the control.

Treatment	Metal Concn ( $\mu\text{M}$ )	SCE/cell $\pm$ SE	IF $\pm$ SE#
$\text{Na}_2\text{CrO}_4$	0	3.57 $\pm$ 0.27	
	0.5	5.33 $\pm$ 0.29	
	1	6.50 $\pm$ 0.51	
	2.5	8.07 $\pm$ 0.44	
	5	(No Metaphase)	
$\text{NiCl}_2$	1	4.07 $\pm$ 0.40	
	0.5+1.0	5.93 $\pm$ 0.31	+0.10 $\pm$ 0.65
	1.0+1.0	5.17 $\pm$ 0.25	-1.63 $\pm$ 0.66
	2.5+1.0	7.80 $\pm$ 0.30	-0.77 $\pm$ 0.68
	5.0+1.0	(No Metaphase)	(N/A)
$\text{Na}_2\text{CrO}_4$ + $\text{NiCl}_2$	5	5.33 $\pm$ 0.37	
	0.5+5.0	6.67 $\pm$ 0.36	+0.067 $\pm$ 0.65
	1.0+5.0	7.00 $\pm$ 0.27	-0.77 $\pm$ 0.74
	2.5+5.0	8.57 $\pm$ 0.42	-0.76 $\pm$ 0.76
	5.0+5.0	10.67 $\pm$ 0.47	(N/A)

Statistical analysis (T-test) indicates a significant increase of SCE frequencies for Ni(II) or Cr(VI) treatments alone ( $p < 0.05$ ). No metaphase have been observed in the cells treated with 5.0  $\mu\text{M}$  of Cr(VI). This observation indicates that there has been a cell cycle arrest during the first replication and the cells have failed to pass the G2 check point due to the DNA damage induced by the high

concentration of Cr(VI). Otherwise, the average SCEs induced by Ni(II) and Cr(VI) alone appears to increase in a dose-dependent manner (graph 1).

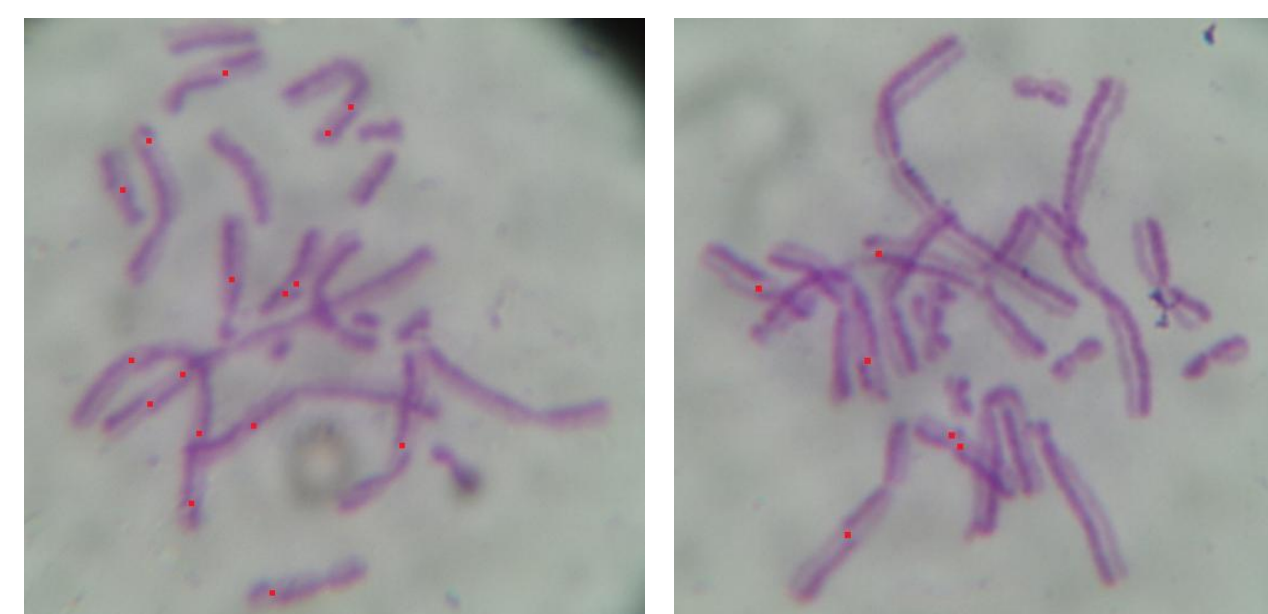


For all the combined treatments a strong sign of antagonism was found, except for 0.5  $\mu\text{M}$  of Cr(VI) treatments combined with 1.0 and 5.0  $\mu\text{M}$  of Ni(II) (Table 1, Graph 1). Although metaphases were observed for the combined treatment of 5.0  $\mu\text{M}$  of Cr(VI) and 5.0  $\mu\text{M}$  of Ni(II), the IF could not be determined due to the absence of data for this particular treatment. However, metaphases and differential staining was observed in the combined treatments, therefore, these findings indicate the completion of two successful rounds of cell replication,

and it may imply an important role of Ni(II) in antagonizing Cr(VI).

For the combined treatment of these metals, the following formula was applied to evaluate the effect of the mixture in the induction of SCE (Katsifis et al;1998):

$IF = \frac{CN - (C - (N + \text{Control}))}{C}$ , where IF is the interaction factor, C is the mean SCE response to Cr(VI), N is the mean SCE response to Ni(II), and CN is the mean SCE response to the mixture of Cr(VI) and Ni(II). A negative IF value indicates an antagonism.



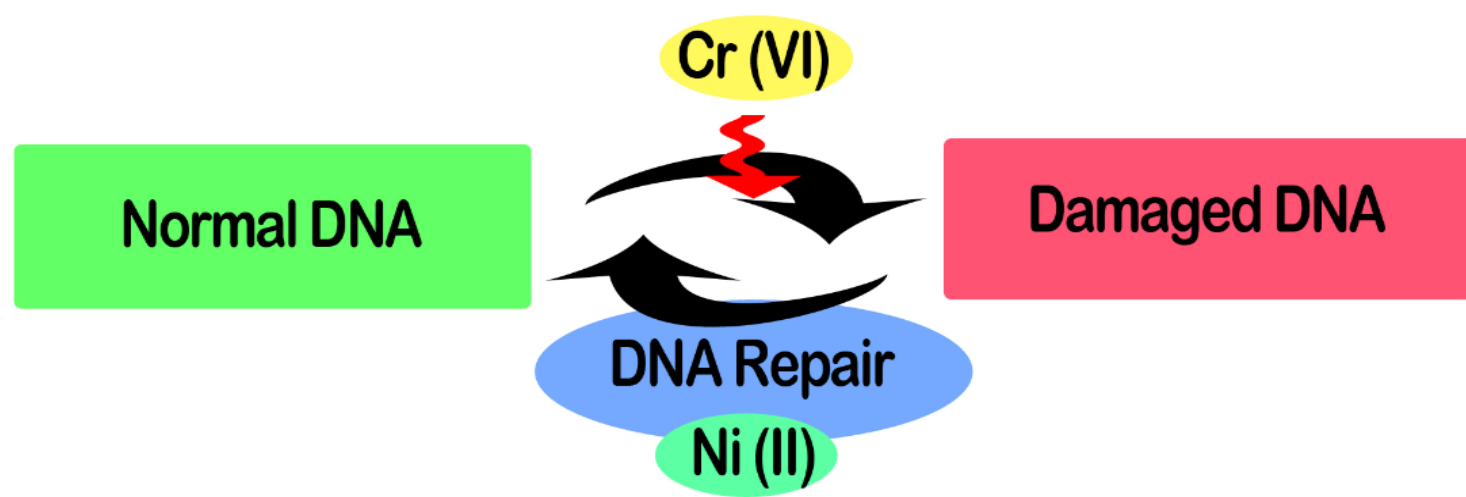
SCE (1.0 Cr(VI))

SCE (1.0 Ni(II)+1.0 Cr(VI))

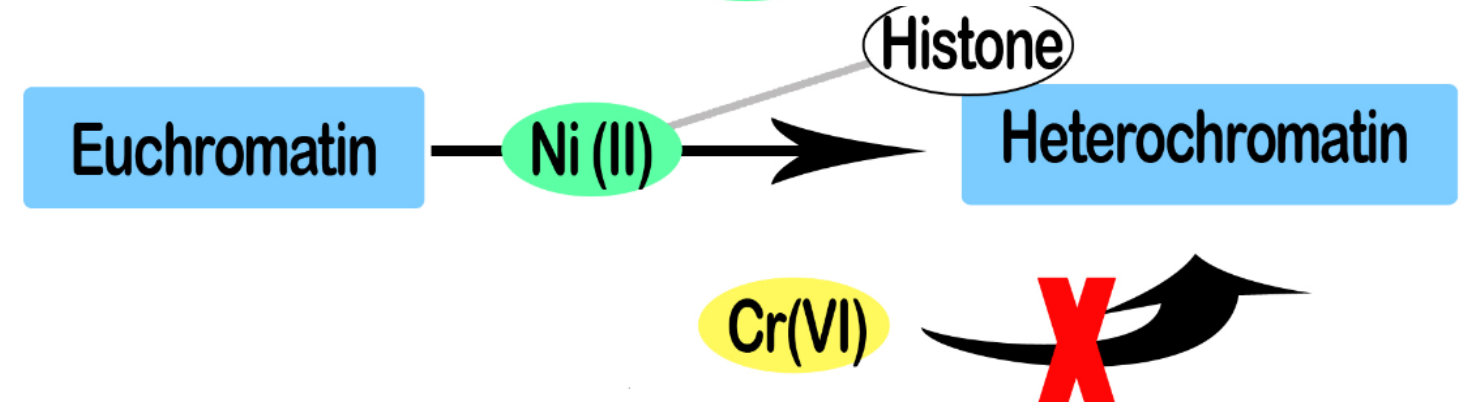
## Conclusion

Antagonistic behavior of nickel and chromium has been demonstrated in this study, and several mechanisms of interaction can be inferred:

1) Ni(II) facilitates DNA repair mechanisms



2) Ni(II) causes linkage between histones. This heterochromatinization can provide steric hindrance for enzymes required for SCE,



3) Ni(II) oxidizes glutathione (GSH  $\rightarrow$  GSSG), causing decreased level of Cr(III), which is the active species of Chromium.



## Future Work

The reduction of Cr(VI) to Cr(III) by GSH in the presence of Ni(II) will be studied. The experiments will test the idea that Ni(II) forms a stable complex with cellular GSH and interferes with the normal inter-conversion between GSH to GSSG, an important process for the reduction of Cr(VI) to its active genotoxic form Cr(III).

These experiments will establish whether the GSH-Ni(II) complex has any effect on the formation of Cr(III) during Cr(VI) reduction through the GSH redox reaction and the subsequent SCE or MN induction.

If the results, however, indicate that the GSH-Ni(II) complex is not likely to be involved in the antagonism of Ni(II) with Cr(VI) for the induction of SCE or MN then this hypothesis will be discarded and testing can proceed on other new hypotheses. More specifically the following will be tested. Ascorbate is another major reducing agent of Cr(VI) to genotoxic form of Cr(III) and GSH is a major protective cellular agent. Furthermore, GSH synthesis has been found to be increased in response to exposures of certain heavy metals. Whether there is an increased GSH synthesis which protects the cells treated by Nickel and Chromium will be studied

## References

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